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ULTRASTRUCTURE OF THE ATYPIC MUSCLES ASSOCIATED WITH TERMINALIAL INVERSION IN MALE *AEDES AEGYPTI* (L)[1]

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Rotation of the male genitalia occurs commonly among the Diptera (reviews by Crampton, 1941; Griffiths, 1972). Two primary types of rotation have been described, a permanent 360° circumversion (Cyclorrapha) and a 180° inversion (most Nematocera and most Brachycera). Among the Nematocera, the 180° inversion may be temporary, occurring only during copulation, or permanent, occurring shortly after adult emergence. Rotation frequently occurs in the intersegmental membrane between abdominal segments VII and VIII, but may involve more than one intersegment of the posterior part of the abdomen.

General reviews of the biology of the Culicidae (Marshall, 1938; Matheson, 1941) suggest that a permanent 180° rotation of the genitalia occurs in all species of this family. Christophers (1915) described inversion of the hypopygium in *Culex fatigans* and later (1922) unsuccessfully attempted to demonstrate abdominal muscles capable of causing rotation. Hodapp (1960) also failed to find muscles suitably positioned to cause rotation in *Aedes aegypti*, although he did experimentally determine that the mechanism for partial rotation was located in the rotating segments themselves.

Fittkau (1971) and Dordel (1973) have recently investigated genitalia rotation in several species of Chironomidae. In contrast to the culicids, both a temporary and a permanent 180° inversion are found among the chironomids. Fittkau and Dordel described complex muscle systems as the rotational mechanism in the posterior part of the abdomen of male chironomids.

The present report is part of an investigation of the rotational process in male *Aedes aegypti*. Ultrastructural changes occurring in the abdominal intersegmental membrane cuticle during rotation are presented in a separate communication (Chevone and Richards, in preparation). This report describes two pairs of opposed muscles found in the rotating region of male *A. aegypti*. These muscles have not been previously reported. The position of these muscles and the histological changes they undergo during rotation indicate that they probably provide the rotational force for inversion of the terminalia.

Material and Methods

A stock of *A. aegypti* (Rutgers strain) was maintained in an environmentally controlled room at 28 ± 1.5° C; 70 ± 10% relative humidity; and a 12 hour photophase. Eggs were vacuum-hatched for 30 minutes (Barbosa and Peters, 1969), and larvae developed in distilled water (250 larvae/liter) fed on dead, dried

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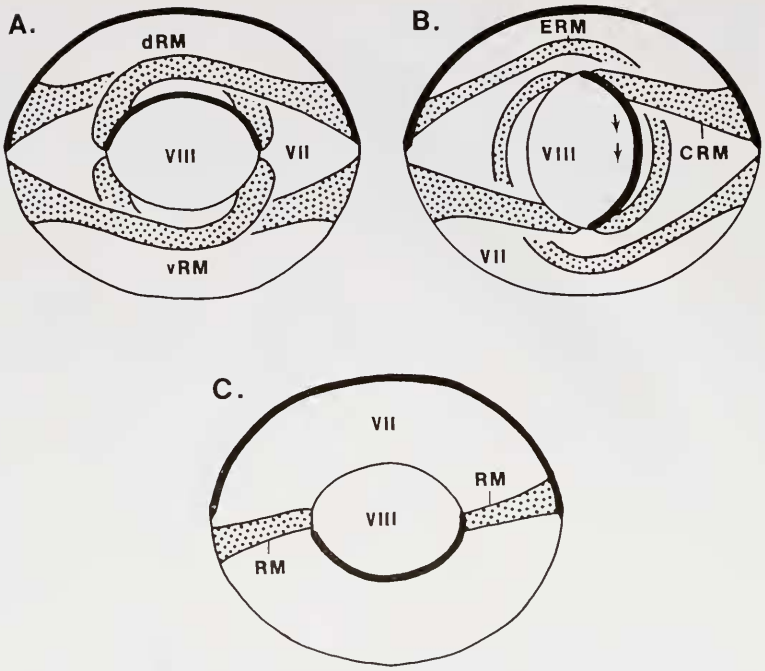

FIGURE 1A-C. Schematic representation of the paired muscles associated with rotation during stages of terminalia inversion. CRM represents contracting rotational muscles; dRM, dorsal rotational muscles; ERM, stretched rotational muscles; RM, muscles producing rotation; vRM, ventral rotational muscles; VII, abdominal segment VII; VIII, abdominal segment VIII.

FIGURE 1A. Cross section of the abdomen through the rotating region in a 32 to 33-hour pharate adult male (48 hours after pupation) showing the pre-rotational position of the paired muscles.

FIGURE 1B. Cross section of the abdomen through the rotating region at 90° of rotation. One member of each muscle set is partially contracted (CRM) and the other has become stretched (ERM). Arrows indicate the direction of rotation.

FIGURE 1C. Cross section of the abdomen through the rotating region 24 hours after the completion of rotation. The stretched muscles are no longer present. The contracted rotational muscles (RM) are positioned to prevent further movement of abdominal segment VIII.

Brewer's yeast. Larval development required 5 to 6 days and pupal development 52 to 75 hours.

For light microscopy, the posterior part of abdomens of males of a known age or degree of rotation were fixed in Dietrich's (Kahle's) fluid. Specimens were dehydrated *via* a graded *n*-butanol-ethanol series (Lee, 1950), embedded in paraffin (mp 61° C) containing 5% beeswax and 5% bayberry wax, and sectioned at 5 or 6 μm. Sections were stained with either Mallory's triple stain or Heidenhain's iron hematoxylin.

For electron microscopy, males of a known age or degree of rotation were first chilled at 10° C for five minutes. Portions of the abdomen (2 to 3 segments each) were fixed at 0 to 4° C for one to two hours in aqueous 2.5% glutaralde-

hyde buffered with 0.06 M phosphate buffer to pH 7.4. Sucrose was added to the fixation to give a final calculated osmolarity of 520 mOsmol. Specimens were then washed for one hour in sucrose-phosphate buffer, post-fixed for four to twelve hours in 1% OsO_4 buffered with 0.06 M phosphate buffer, dehydrated *via* an ethanol-propylene oxide series, and embedded in Epon 812. Sections were cut on a Reichert ultramicrotome at 80 to 100 nm, stained with lead citrate and uranyl acetate, and examined in a Philips 300 transmission electron microscope at 60 kV.

Results

General aspects of rotation

Rotation of the male terminalia was observed at 28° C and found to correspond with Hodapp's (1960) report. Rotation begins 1 to 4 hours after adult emergence and reaches 90° in 6 to 12 hours after emergence. A variable resting period of 1 to 6 hours occurs at the 90° position and rotation is completed in 18 to 24 hours. Rotation may be either clockwise or counterclockwise in a 1:1 ratio (66:62, respectively, in specimens tabulated in this study).

Light microscopy of atypic muscles associated with terminalia rotation

In cross sections of pharate adults (42 to 48 hours after pupation) or newly emerged male imagoes, two sets of opposed, crossed muscles, one set dorsal and one set ventral, are present in the rotating intersegmental region (Figs. 1A and 2). Similar muscles were not found in the few female specimens that were examined.

Electron microscopy shows that each of these muscles in the male is composed of 10 to 12 fibers. The muscles originate anteriorly, on the posterior lateral margins of the sclerites of abdominal segment VII. They cross the intersegmental membrane obliquely and insert on the anterior lateral margins of the sclerites of abdominal segment VIII. The origin of each muscle is on the opposite side of the body from the insertion; this results in each muscle pair being crossed.

The origins of these paired muscles are quite distinct in paraffin sections, and appear localized at the posterior lateral margins of the sclerites of segment VII (Figs. 2 and 3). The insertions on segment VIII are more difficult to observe, however. This is primarily due to the close apposition of the intersegmental membrane against the sclerite cuticle of abdominal segment VII in the area where segment VIII is retracted within segment VII. The exact position of the insertions is, therefore, unclear, and they may extend to some degree circumferentially along the sclerite-intersegmental membrane junction.

Prior to the onset of rotation, both muscles of a set are of equal length. As rotation proceeds, one muscle of each set shortens and the opposed one becomes elongated (Fig. 1B). The muscles that contract remain taut, from origin to insertion, throughout the majority of the rotational process (Fig. 3). In the final 10 to 20° of rotation, bending and folding of some individual fibers occurs to a limited extent.

Upon completion of rotation, at 24 hours after emergence of the adult, both contracted and stretched muscles are present. At 48 hours, the stretched muscles can no longer be found, and only 2 to 4, rather than 10 to 12, contracted muscle fibers are present. The insertion of the contracted fibers which remain, are now

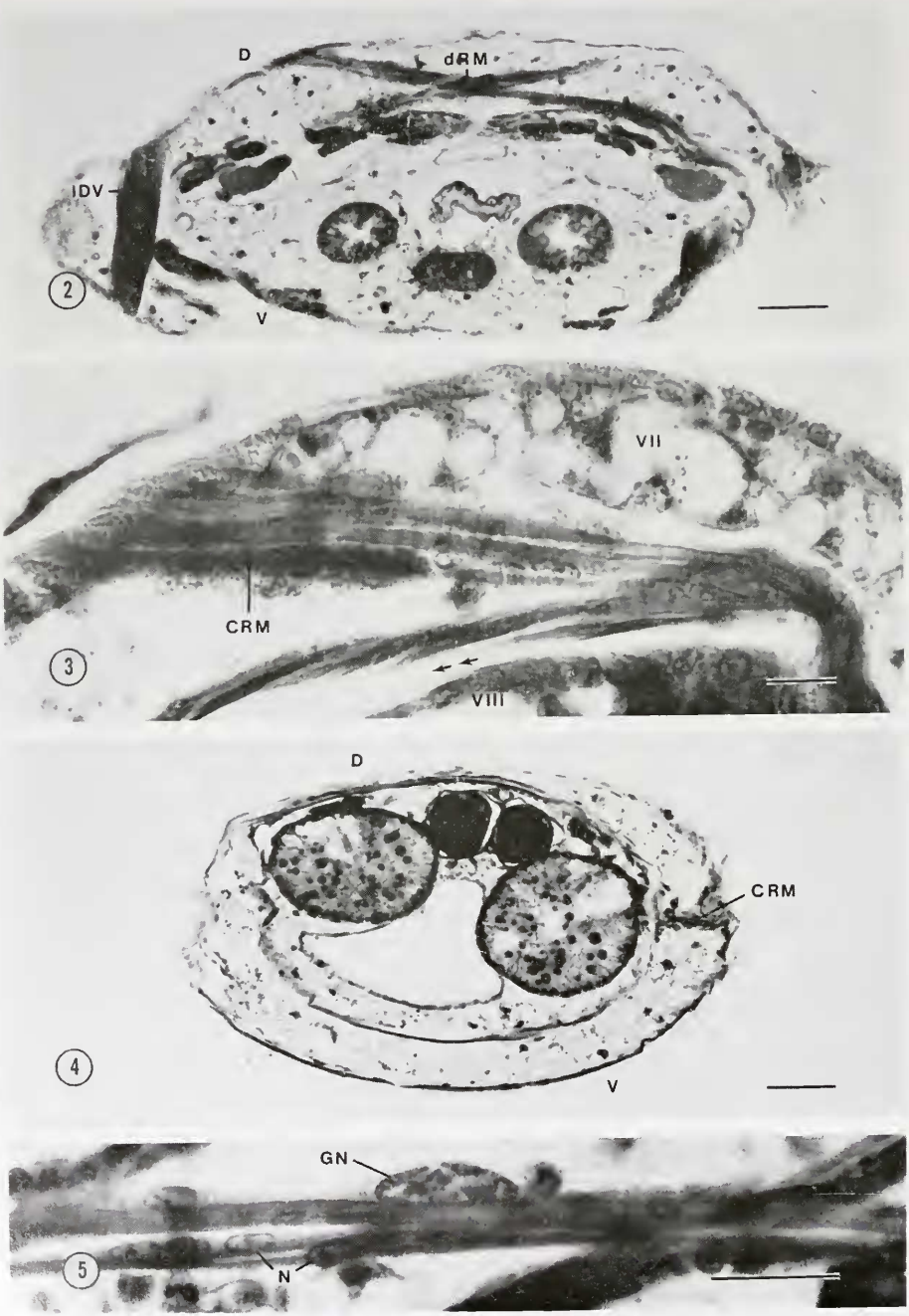

FIGURE 2. Cross section of the abdomen through the rotating region of a 26 to 27-hour pharate adult (42 hours after pupation). Compare with Figure 1A. The position of the dorsal rotational muscles is shown prior to rotation (hematoxylin). D represents dorsum

localized at the lateral, anterior margins of the sclerites of abdominal segment VIII (Figs. 1C and 4). The contracted muscles persist for at least the first week of adult life (older adults were not examined).

Characteristic of each muscle is a single giant nucleus, three to four times larger in length than the normal muscle nuclei (Figs. 5 and 6). These giant nuclei first appear in the 2 to 3-hour pharate adult (18 hours after pupation), persist until rotation is completed, and then can no longer be found.

Rotation involves an unusual reduction in the length of the contracting muscles. The muscle length is approximately 300 μm in the 26 to 27-hour pharate adult (42 hours after pupation), whereas the average length of the contracted muscle is only 69 μm in the 48-hour adult. This is equivalent to a 75 to 80% reduction during contraction. Correspondingly, the stretched muscles become elongated from 300 μm to approximately 2.5 times this length during rotation. Banding in the pre-rotational muscles is diffuse (Figs. 5 and 12), and only the A- and I-bands are apparent.

The crossed muscles are not present in the early pupa. They first appear as columns of aggregated myoblasts in the 2 to 3-hour pharate adult (18 hours after pupation); however, their position is quite unlike that in the newly-emerged adult. The insertions of the presumptive muscles are in the same general position as in the adult, near the anterior lateral margins of the eight abdominal sclerites. The myoblast columns follow the contours of the eighth abdominal sclerite to about the mid-sclerite position (tergite or sternite), whereupon they project vertically to the posterior mid-sclerite margins of the seventh abdominal segment (Fig. 6). They do not attach to the sclerites, but terminate just below the epidermis. A narrow zone of granular material, staining intensely blue with Mallory's stain (electron micrographs show this zone to consist of basement membrane), connects the myoblast columns and the basal region of the epidermal cell layer.

In the 8 to 9-hour pharate adult (24 hours after pupation), the myoblast columns have elongated and become thinner (Fig. 7). The crossed structure has become more apparent and the terminal regions (origins) beneath the epidermis of the seventh abdominal segment have begun to extend laterally.

In the 14 to 15-hour pharate adult (30 hours after pupation), the origins of the myoblast masses have begun to separate. They do so rapidly, the presumptive muscles reaching their approximate adult positions within 12 hours (Fig. 2).

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of abdomen; dRM, dorsal rotational muscles; lDV, lateral dorso-ventral muscle; V, venter of abdomen; bar equals 50 μm.

FIGURE 3. Partially contracted rotational muscle at 90° of rotation. Compare with Figure 1C. The individual fibers composing the muscle are evident. The arrows indicate the direction of rotation (Mallory's triple strain). CRM represents contracting rotational muscle; VII, abdominal segment VII; VIII, abdominal segment VIII; bar equals 20 μm.

FIGURE 4. Cross section through the abdomen of the rotating region in a 48-hour adult male, 24 hours after the completion of rotation. Compare with Figure 1D. Only one of the contracted rotational muscles is apparent in this section (hematoxylin). CRM represents contracted rotational muscle; D, dorsum of abdomen; V, venter of abdomen; bar equals 50 μm.

FIGURE 5. Fibers of the rotational muscles prior to rotation in the 26 to 27-hour pharate adult. Diffuse banding is visible in one of the fibers. The comparative sizes of the giant nucleus and the typical muscle nuclei are evident (hematoxylin). GN represents giant nucleus; N, typical muscle nuclei; bar equals 20 μm.

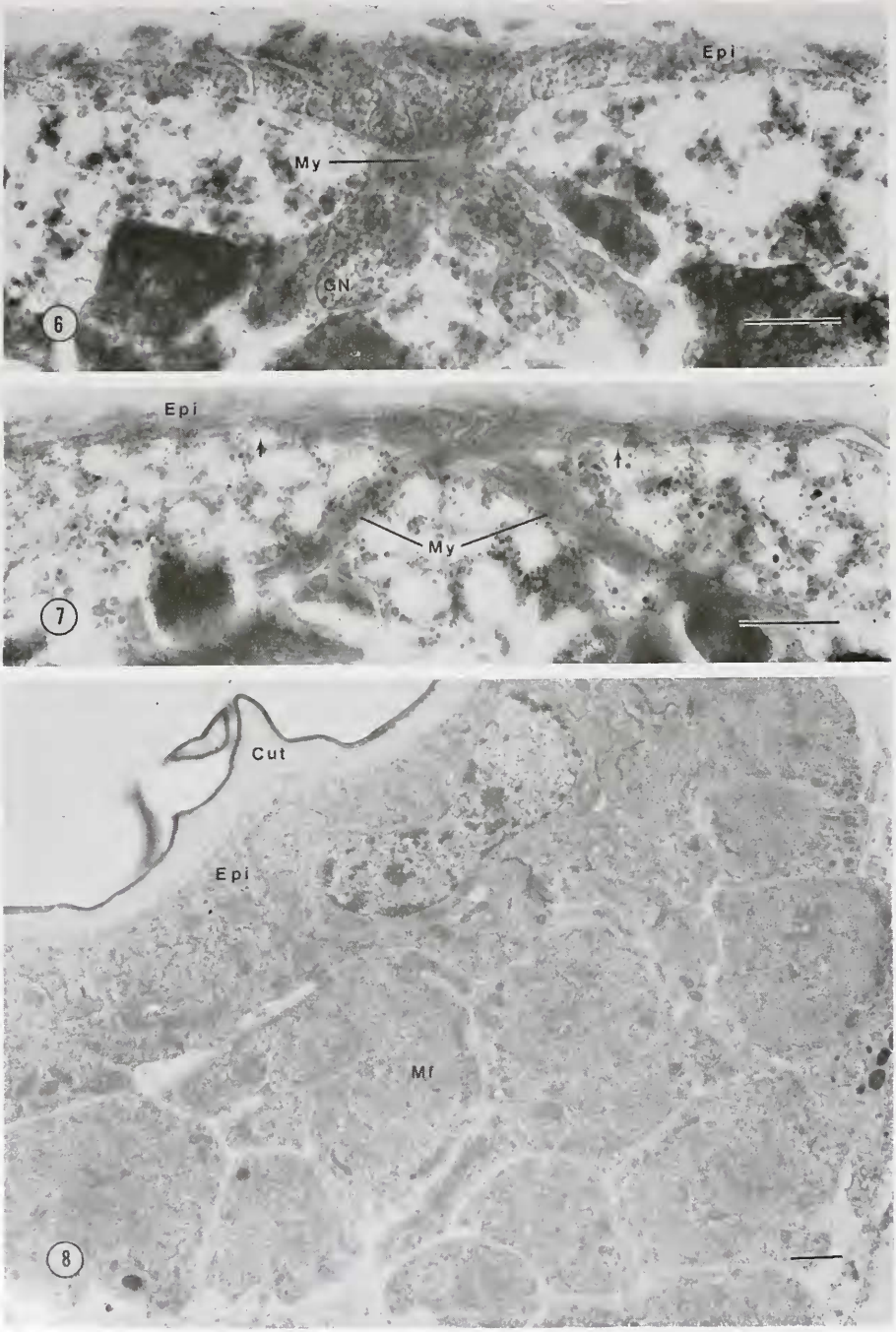

FIGURE 6. Developing rotational muscles in the 2 to 3-hour pharate adult (18 hours after pupation). The presumptive muscles appear as columns of aggregated myoblasts at this stage.

Muscle banding first becomes visible in specimens stained with hematoxylin at this time (Fig. 5).

Ultrastructure of the atypic muscles associated with rotation

The ultrastructure of the rotational muscle was examined at four stages: prior to rotation (1) in the pharate adult (42 or 48 hours after pupation), and (2) in the newly emerged adult (0 to 2 hours); (3) at 90° of rotation; and (4) at one day after rotation (48 hours post emergence).

The subdivision of each muscle into 10 to 12 fibers is evident in cross sections of the pre-rotational muscles 48 hours after pupation (Fig. 8). The muscle fibers are generally round to oval in cross section and range in diameter from 10 to 40 μm. A thin, granular basement membrane, about 22 nm thick, invests each muscle fiber. The entire fiber group, however, is not encased in a separate extracellular sheath. The fibers closely adjoin each other and the basement membranes of adjacent fibers are separated by 100 to 200 nm (Figs. 9 and 11).

The structural organization of the contractile elements can be seen in near longitudinal section in Figure 12. The muscle fibers are not separated into distinct myofibrils, and sarcoplasmic organelles appear randomly dispersed within the contractile fields. The muscle fibers are subdivided along their length into sarcomeres by Z-lines. A-bands and I-bands are the only distinguishable regions within the sarcomeres. The junction of the A- and I-bands is not clearly defined, as some of the thick filaments extend into the I-band various distances. The Z-lines are irregular and appear as discontinous patches across the muscle fibers (Figs. 12 and 16).

The sarcomere length in these muscles, prior to rotation, ranges from 6.8 to 8.3 μm. The A-band averages 3.7 μm; the I-band, 3.4 μm; and the Z-line ranges from 185 to 230 nm. These values are comparable to those of other insect slow muscles (Hagopian, 1966; Smith, Gupta and Smith, 1966; Osborne, 1967; Crossley, 1968).

The internal membrane system in these muscles is not very extensive. Invaginations of the plasma membrane to form the T-system are sparse and appear to penetrate only about a micrometer into the muscle fiber (Fig. 9). The sarcoplasmic reticulum is not abundant and dyads are uncommon. Mitochondria are sparse, and oval to slightly elongate, with well-developed cristae. They appear randomly distributed throughout the muscle fiber (Figs. 8 and 9). Numerous electron dense granules (glycogen?) are found in close association with the mitochondria.

Cross sections of the muscle fibers show a thick-thin (myosin-actin) filament ratio of about 1:5 (Fig. 10), 10 to 12 thin filaments surrounding each thick fila-

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The terminal ends of the columns (origins) are not attached to the epidermis and are located along the mid-line of the presumptive 7th segment sclerites rather than at the lateral margins of the sclerites as in the newly-emerged adult (Mallory's triple stain). Epi represents epidermis; GN, giant nucleus; My, myoblast column; bar equals 20 μm.

FIGURE 7. Developing rotational muscles in the 8 to 9-hour pharate adult (24 hours after pupation). The myoblast columns have elongated and become thinner, and the origins (arrows) have begun to extend laterally (Mallory's triple stain). Epi represents epidermis; My, myoblast column; bar equals 20 μm.

FIGURE 8. Cross section of a rotational muscle in the 26 to 27-hour pharate adult (42 hours after pupation). The subdivision of the muscle into 10 to 12 fibers is apparent. Cut represents cuticle; Epi, epidermis; Mf, muscle fiber; N, nucleus; bar equals 1.0 μm.

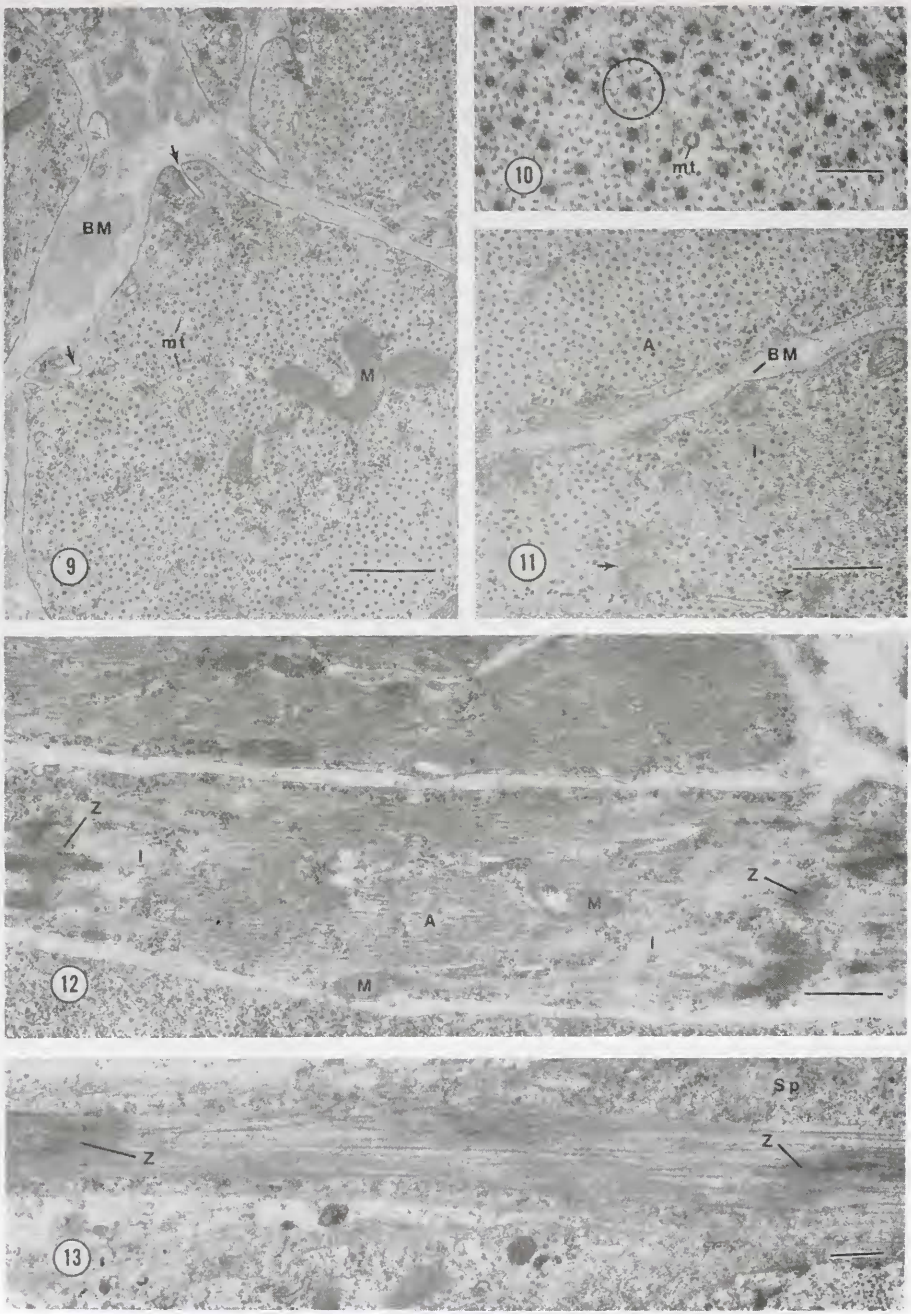

FIGURE 9. Cross section of a portion of a rotational muscle in a 26 to 27-hour pharate adult (42 hours after pupation). A thin basement membrane surrounds each muscle fiber. Invaginations of the plasmalemma (arrows) are sparse and do not penetrate deeply into the

ment. The thick filaments are 17 to 20 nm in diameter and thin filaments, 4 to 5 nm. The center-to-center spacing of the thick filaments is 55 to 60 nm. Many of the thick filaments have hollow central cores (Fig. 10). Numerous microtubules, 22 to 25 nm in diameter, are present scattered throughout the contractile fields (Fig. 10).

At 90° of rotation, three to six hours after muscle contraction is evident, one of the opposed muscles in each set is partially stretched and the other partially contracted. Longitudinal sections of a stretched fiber show a sarcomere length of 8.5 to 11.4 μm. Thick and thin filaments span the entire sarcomere, and no I-band is present (Fig. 13). The Z-lines appear more diffuse than in the pre-rotational condition and have become elongated along the axis of the muscle fiber to a width of 1.0 to 1.4 μm (Fig. 17).

Two types of contracting muscle fibers are distinguishable at the 90° rotation stage, apparently corresponding to different degrees of contraction. In one type, the sarcomere length has shortened to 3.9 to 4.5 μm,, or 45% less than in the uncontracted state. The I-band is still present (Figs. 14 and 18), and ranges from 0.18 to 0.23 μm, accounting for nearly the entire reduction in sarcomere length. All myofilaments in this fiber type parallel the long axis of the muscle. In the other type of contracting fiber (Fig. 15), the sarcomeres have an average length of 3.4 μm, or 55% less than in the uncontracted state; no I-band is present and the thick filaments penetrate to the Z-line. Many of the myofilaments are parallel to the long axis of the muscle fiber; however, some sarcomeres have their myofilaments arranged at various angles, up to 90°, to the longitudinal axis. This multidirectional arrangement of myofilaments within a muscle fiber has been previously noted by Rice (1970) in the super-contracting oesophageal muscles of the Tsetse fly, by Elder (1975) in the flight control muscle of *Vespa* and by Devine and Somolyo (1971) in vertebrate smooth muscle. The Z-banding in these two types of contracting fibers is quite different. In the unidirectional fibers (Fig. 14), the Z-lines are nearly continuous across the fibrils and are in fairly regular register. In the multidirectional fibers, most of the Z-lines are incomplete and there is poor alignment across the fibers (Fig. 15). In both types of muscle fiber, however, the Z-lines have condensed considerably from the pre-rotational condition, and are 69 to 115 nm in width (originally 185 to 230 nm).

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fibers. BM represents basement membrane; M, mitochondria; mt, microtubules; bar equals 0.5 μm.

FIGURE 10. Arrangement of thick and thin filaments in a rotational muscle fiber. Each thick filament is surrounded by 10 to 12 thin filaments, maximally clear in circle. Microtubules are present in the contractile fields. Mt represents microtubules; bar equals 0.1 μm.

FIGURE 11. Cross section of rotational muscle fibers in a newly emerged adult. I-band regions (I), composed predominately of thin filaments, are not entirely devoid of thick filaments. Electron dense patches (arrows) are probably portions of Z-bands. A represents A-band; BM, basement membrane; I, I-band; bar equals 0.5 μm.

FIGURE 12. Nearly longitudinal section of rotational muscle fibers in a 32 to 33-hour pharate adult (48 hours after pupation). The irregular Z-bands (Z) limit one sarcomere. The definition between the A-band and the I-band is indistinct. Sarcoplasmic organelles appear randomly dispersed within the contractile fields. A represents A-band; I, I-band; M, mitochondria; Z, Z-band; bar equals 0.5 μm.

FIGURE 13. Longitudinal section of a stretched muscle fiber at 90° of rotation. The Z-bands limiting the sarcomere are elongated along the fiber axis. Thick filaments span the entire sarcomere. Sp represents sarcoplasm; Z, Z-band; bar equals 0.5 μm.

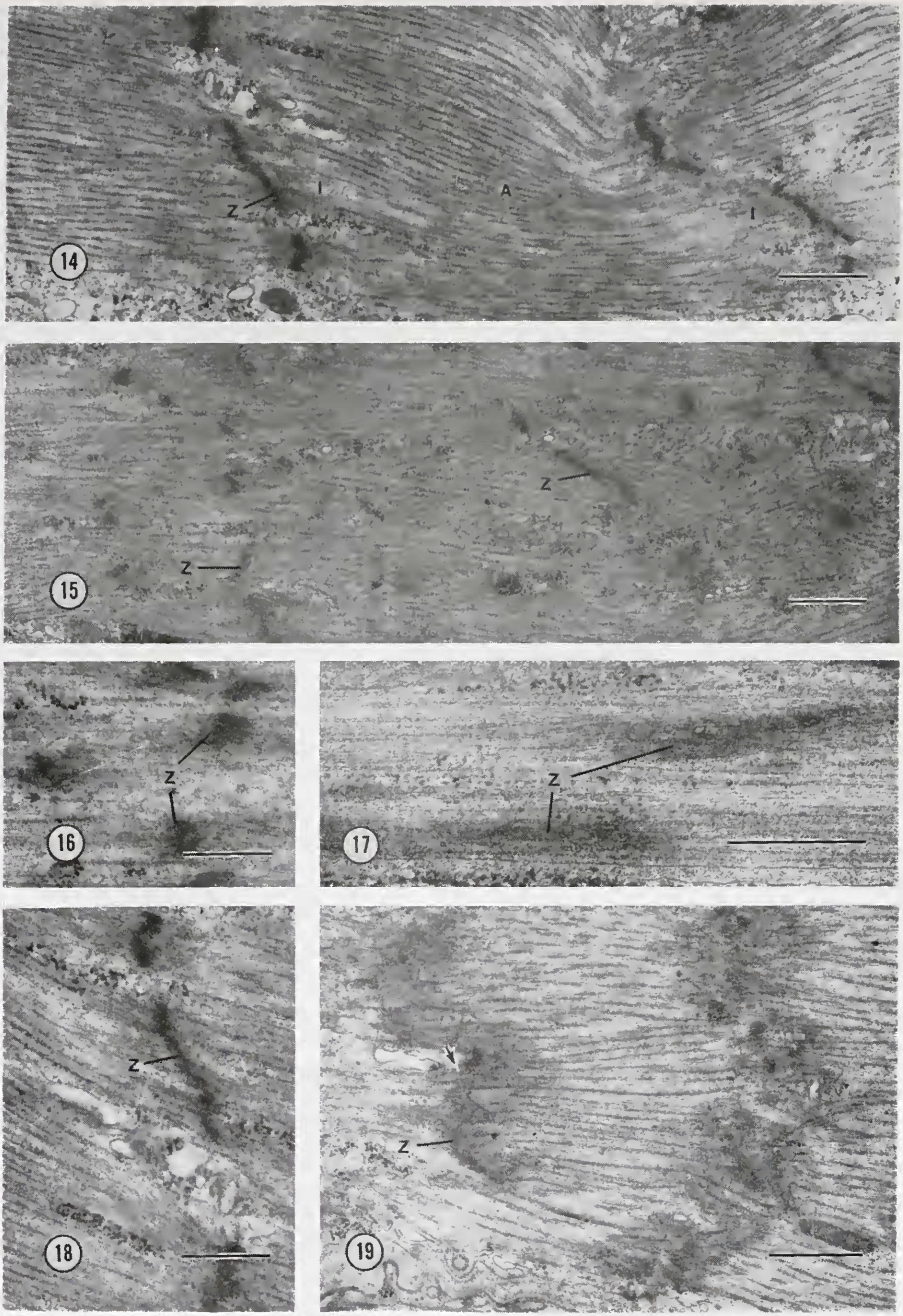

FIGURE 14. Rotational muscle contracted about 45% at 90° of rotation. A narrow I-band is still present. The Z-bands are condensed and fairly regular across the fibril. A represents A-band; I, I-band; Z, Z-band; bar equals 0.5 μm.

The partially contracted muscles appear structurally more organized in comparison to the uncontracted muscles. The appearance of the myofibrils has become somewhat more distinct and the sarcoplasmic reticulum and electron dense granules are now found principally concentrated between the myofibrils and along the Z-lines (Figs. 14 and 18). Mitochondria are generally restricted to the sarcoplasm surrounding the contractile elements and are not found within the myofilament fields.

The fully contracted muscle in the 48-hour adult has sarcomere lengths of 1.6 to 1.9 μm, a 75% reduction over that in the uncontracted muscle; this corresponds to the 75 to 80% reduction in total muscle length. There is no I-banding present and thick filaments can occasionally be seen passing through discontinuities in the Z-lines (Fig. 19). The Z-lines are less distinct than at 90° of rotation. They remain condensed, however, having a width of 69 to 90 nm. The cellular organelles are now primarily located between the myofibrils, and there is little sarcoplasm present between the sarcolemma and the contractile fields (Fig. 19). Mitochondria and electron dense granules are in close association with the Z-lines.

Discussion

Two sets of opposed, crossed muscles are present in the rotating intersegmental region in the newly-emerged male adult of *A. aegypti*. These muscles were overlooked by Christophers (1922) and Hodapp (1960) in their investigations of the rotational mechanism.

The histological appearance of these muscles during terminalia rotation, while not conclusive evidence in itself, strongly suggests that they function as the driving force for rotation. The muscles are positioned to exert force in a plane nearly perpendicular to the long axis of the abdomen, the plane in which rotation occurs. The contracting muscles remain taut during rotation; they do not appear to be passively dragged along as do the longitudinal intersegmental muscles (Christophers, 1922; Hodapp, 1960). The crossed structure allows for rotation in either a clockwise or counterclockwise direction, and also probably aids in stabilizing the rotational axis along the longitudinal body axis. The contracted muscles, upon completion of rotation, are positioned to prevent further movement of the eighth segment relative to the seventh.

If the crossed muscles are solely responsible for rotation (Hodapp, 1960 and Jones, 1968, have suggested that periodic contractions of the hindgut function as

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Figure 15. Rotational muscle contracted about 55% at 90° of rotation. Thick filaments span the entire sarcomere and there is no I-band present. The myofilaments do not all parallel the long axis of the muscle fiber. Z-bands are incomplete and irregular across the fibers. Z represents Z-bands; bar equals 0.5 μm.

Figure 16. Z-band in the pre-rotational muscle. The Z-band appears as discontinuous beads to which the thin filaments attach. Z represents Z-band; bar equals 0.5 μm.

Figure 17. Z-band in the stretched rotational muscle at 90° of rotation. The Z-band is diffuse and elongated. Thin filaments appear to pass through the Z-band material. Z represents z-band; bar equals 0.5 μm.

Figure 18. Z-band in a partially contracted muscle at 90° of rotation. The Z-bands are condensed. Z represents Z-band; bar equals 0.5 μm.

Figure 19. Contracted rotational muscle 24 hours after the completion of rotation. Thick filaments span the entire sarcomere and can occasionally be seen passing through discontinuities (arrow) in the Z-band. Z represents Z-band; bar equals 0.5 μm.

the driving force), then gross contraction characteristics can be inferred from movement of the terminalia during rotation. Hodapp (1960) described movement as occurring "by a series of strong, twisting motions" followed by variable quiescent periods, the one at 90° of rotation being the longest, lasting from one to six hours. These overt contraction characteristics, however, are presumably modifications produced by numerous other factors in the system. Although the muscles appear histologically functional in the pharate adult 48 hours after pupation, initial movement of the terminalia does not take place until one to four hours after adult emergence. The initial 90° of rotation requires only about three hours, whereas the final 90° requires about seventeen hours. There is a prolonged cessation of movement (contraction?) at 90° of rotation. The above characteristics of rotation are functions of the entire system and additional information is necessary to understand them. Nevertheless, with the data available, the muscles which contract appear atypic in gross physiological characteristics. They contract 75 to 80% of their original length, and this requires a period of 18 to 24 hours. In addition, these muscles display the unusual property of shortening only once. Upon the completion of rotation, the two to four contracted muscle fibers which persist appear to maintain a state of prolonged contraction.

It is interesting that the member of the muscle pair which becomes elongated (presumably stretched) never shows any conclusive evidence of contraction and soon disappears. We know of no other report of a muscle that never shortens. Whether it is never stimulated, or inhibited by the stretching, or overcome by its opponent, or affected in some other way, it degenerates and disappears without ever having shortened.

Since rotation can occur either clockwise or counterclockwise in a 1 : 1 ratio, it appears that, at random, one or the other member of a set starts to contract, or contracts more strongly, thereby determining the direction of rotation. We have no evidence as to how this is effected.

Although the rotational muscles supercontract to 75 to 80% of their original length, they possess only limited ultrastructural similarities to previously described supercontracting muscles in the Diptera (Osborne, 1967; Rice, 1970; Goldstein and Burdette, 1971; Crossley, 1968, 1972; Pringle, 1972; Elder, 1975). The rotational muscles in *A. aegypti*, especially prior to rotation, have Z-lines that appear perforated in longitudinal section. However, the characteristic cross sectional profiles of perforated Z-discs (as shown by Osborne, 1967, and Crossley, 1968) were not found. In addition, beyond 50% contraction, although a few thick filaments were found passing through discontinuities in the Z-lines of contracting fibers, this occurrence was much less than that found by other investigators. In *A. aegypti*, contraction of the rotational muscles beyond 50% appears to involve primarily a folding or overlapping of thick filaments adjacent to the Z-lines, rather than a sliding of thick filaments through perforated Z-discs.

Other ultrastructural features of the rotational muscles are typical of slow contracting muscles. The organization of these into incompletely separated myofibrils and the poor alignment of the contractile elements across the fibers are also found in other insect slow skeletal and visceral muscles (Rice, 1970; Crossley, 1968, 1972). This is in contrast to 'fast' fibrillar flight muscle in which the myofibrils are distinctly separated and the sarcomeres are in perfect register (Smith, 1966). A thick/thin filament ratio of 1 : 5 is characteristic of slow skeletal and visceral

muscles, whereas rapidly contracting flight muscle has a 1 : 3 ratio. Auber (1967) has correlated a high thin/thick filament ratio with a slow work rhythm, and Goldstein (1971) has suggested that the greater number of filaments may aid in maintaining tension for prolonged periods.

The internal membrane system of muscles has been correlated with the excitation-contraction coupling and the contraction-relaxation phase in insects (Huddart and Oates, 1970; Tyrer, 1973). The poorly-developed sarcoplasmic reticulum and the low number of dyads and T-system tubules in the pre-rotational muscles in *A. aegypti* are consistent with the slow contraction rhythm of insect muscles of similar appearance.

The rotational mechanism of terminalia inversion has been reported in only a a few species of Diptera. Fittkau (1971) and Dordel (1973) have described the muscles involved in rotation in several species of chironomids, and the present study is the first report of the rotational musculature in a culicid. In the Chironomidae, rotation may be either temporary or permanent (Fittkau, 1971), and in *Clunio marinus*, a permanent 180° rotation occurs within two hours of adult emergence and involves three abdominal segments (Dordel, 1973). The two families, Chironomidae and Culicidae, are considered to be closely related phylogenetically, yet the rotational mechanism described in species of these two families is considerably different both morphologically and physiologically. Further studies of the inversion mechanism in other lower Diptera displaying a 180° rotation of the genitalia should result in interesting comparative relationships.

Summary

Two sets of opposed, crossed muscles are present in the rotating region of the abdomen in male *A. aegypti*. These muscles undergo changes during rotation of the genitalia that suggest they function as the driving force for rotation. During this rotation, one muscle of each set contracts and the opposed one becomes elongated.

The contracting muscles are atypic physiologically. They contract from 300 μm to about 69 μm, and this requires a period of 18 to 24 hours. They shorten only once and those muscle fibers still present after the completion of rotation remain in a contracted condition at least for two weeks. The elongated muscles never shorten; they become stretched to approximately 2.5 times their original length and disappear soon after rotation is completed.

LITERATURE CITED

Auber, J., 1967. Distribution of two kinds of myofilaments in insect muscles. *Amer. Zool.*, **7**: 451–456.

Barbosa, P. and T. M. Peters, 1969. A comparative study of egg hatching techniques for *Aedes aegypti* (L). *Mosquito News*, **29**: 548–551.

Christophers, S. R., 1915. The male genitalia of *Anopheles*. *Indian J. Med. Res.*, **3**: 371–394.

Christophers, S. R., 1922. The development and structure of the terminal abdominal segments and hypopygium of the mosquito with observations on the homologies of the terminal segments of the larvae. *Indian J. Med. Res.*, **10**: 530–572.

Crampton, G. C., 1941. The external morphology of the Diptera. *Bull. Conn. Geol. Natur. Hist.*, **64**: 10–165.

Crossley, A. C., 1968. The fine structure and mechanism of breakdown of larval intersegmental muscles in the blowfly *Calliphora erythrocephala*. *J. Insect Physiol.*, **14**: 1389–1407.

Crossley, A. C., 1972. Ultrastructural changes during transition of larval to adult intersegmental muscle at metamorphosis in the blowfly *Calliphora erythrocephala* (Dipt. Calliphoridae). II. The formation of adult muscles. *J. Embryol. Exp. Morphol.*, **27**: 75–101.

Devine, C. E. and A. P. Somlyo, 1971. Thick filaments in vascular smooth muscle. *J. Cell. Biol.*, **49**: 636–649.

Dordel, H. J., 1973. Funktionsanatomische Untersuchungen über die Abdomentorsion bei der männlichen Imago von *Chunio marinus* Haliday (Diptera, Chironomidae). *Z. Morphol. Tiere*, **75**: 165–221.

Elder, H. Y., 1975. Muscle structure. Pages 1–74 in P. N. R. Usherwood, Ed., *Insect muscle*, Academic Press, London, New York.

Fittkau, E. J., 1971. Der Torsions-Mechanismus bei Chrionomiden-Hypopygium. *Limnologica*, **8**: 27–43.

Goldstein, M. A., 1971. An ultrastructural study of super-contraction in the body wall muscles of *Drosophila melanogaster* larvae. *Anat. Rec.*, **169**: 326.

Goldstein, M. A. and W. J. Burdette, 1971. Striated visceral muscle of *Drosophila melanogaster* (Dipt. Drosophilidae). *J. Morphol.*, **134**: 315–334.

Griffiths, G. C. P., 1972. *The phylogenetic classification of Diptera Cyclorrhapha*. Dr. W. Junk, N.V., The Hague, 340 pp.

Hagopian, M., 1966. The myofilament arrangement in the femoral muscle of the cockroach, *Leucophea maderae* Fabricius. *J. Cell. Biol.*, **28**: 545–562.

Hodapp, C. J., 1960. The mechanism of terminalia rotation in male *Aedes aegypti* (L). *Masters thesis, University of Maryland*, College Park, Maryland, 43 pp.

Huddart, H. and K. Oates. Ultrastructure of stick insect and locust skeletal muscle in relation to excitation-contraction coupling. *J. Insect Physiol.*, **16**: 1467–1483.

Jones, J. C., 1968. The sexual life of a mosquito. *Sci. Amer.*, **218**: 108–116.

Lee, B., 1950. Dehydration. Pages 61–62 in J. B. Gatenby and H. W. Beams, Eds., *The microtomist's vade-mecum*, 11th Edition. The Blakiston Co., Philadelphia.

Marshall, J. F., 1938. *The British mosquitoes*. W. Clowes and Sons, Ltd. London, 341 pp.

Matheson, R., 1941. *Handbook of the mosquitoes of North America*. Comstock Publishing Co. Inc., Ithaca, New York, 314 pp.

Osborne, M. P., 1967. Supercontraction in the muscles of the blowfly larva: an ultrastructural study. *J. Insect Physiol.*, **13**: 1471–1482.

Pringle, J. W. S., 1972. Arthropod muscle. Pages 491–561 in G. H. Bourne, Ed., *The structure and function of muscle*, Vol. 1, Part I, 2nd Ed. Academic Press, New York.

Rice, M. J., 1970. Supercontracting and non-supercontracting visceral muscles in the Tsetse fly, *Glossina austeni*. *J. Insect Physiol.*, **16**: 1109–1122.

Smith, D. S., 1966. The organization of flight muscle fibers in the Odonata. *J. Cell Biol.*, **28**: 109–126.

Smith, D. S., B. L. Gupta and U. Smith, 1966. The organization and myofilament array of insect visceral muscles. *J. Cell Sci.*, **1**: 49–57.

Tyrer, N. M., 1973. Functional development of the reticular system in an insect with synchronously differentiating cells. *J. Cell Sci.*, **12**: 197–215.